NEW ENGLAND BioLabs® Inc.

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New England Biolabs
Product Specification

Product Name:	*EagI-HF®*
Catalog #:	*R3505S/L*
Concentration:	*20,000 units/ml*
Unit Definition:	*One unit is defined as the amount of enzyme required to digest 1 µg of pXba DNA in rCutSmart Buffer in 1 hour at 37°C in a total reaction volume of 50 µl.*
Shelf Life:	*24 months*
Storage Temp:	*-80°C*
Storage Conditions:	*500 mM NaCl, 10 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol, 200 µg/ml rAlbumin, (pH 7.4 @ 25°C)*
Specification Version:	*PS-R3505S/L v4.0*
Effective Date:	*18 Dec 2023*

Assay Name/Specification (minimum release criteria)
Blue-White Screening (Terminal Integrity) - A sample of Litmus38i vector linearized with a 10-fold excess of EagI-HF™, religated and transformed into an *E. coli* strain expressing the LacZ beta fragment gene results in <1% white colonies.
Endonuclease Activity (Nicking) - A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 20 units of EagI-HF® incubated for 4 hours at 37°C results in <20% conversion to the nicked form as determined by agarose gel electrophoresis.
Exonuclease Activity (Radioactivity Release) - A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA and a minimum of 100 units of EagI-HF® incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.
Functional Testing (15 minute Digest) - A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of pXba DNA and 1 µl of EagI-HF® incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.
Ligation and Recutting (Terminal Integrity) - After a 20-fold over-digestion of pXba DNA with EagI-HF™, >95% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, >95% can be recut with EagI-HF™.
Non-Specific DNase Activity (16 Hour) - A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of pXba DNA and a minimum of 100 units of EagI-HF® incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.
Protein Purity Assay (SDS-PAGE) - EagI-HF™ is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection.

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qPCR DNA Contamination (*E. coli* Genomic) - A minimum of 20 units of EagI-HF® is screened for the presence of *E. coli* genomic DNA using SYBR® Green qPCR with primers specific for the *E. coli* 16S rRNA locus. Results are quantified using a standard curve generated from purified *E. coli* genomic DNA. The measured level of *E. coli* genomic DNA contamination is ≤ 1 *E. coli* genome.

Nancy Considine
Quality Approver

Date 18 Dec 2023